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DOI:
10.1016/j.neuroscience.2016.10.006
10.1016/j.neuroscience.2016.10.006

Document Version
Peer reviewed version

Link to publication record in King's Research Portal

Citation for published version (APA):
Tomimatsu, Y., Cash, D., Suzuki, M., Suzuki, K., Bernanos, M., Simmons, C., Williams, S. C. R., & Kimura, H. (2016). TAK-063, a phosphodiesterase 10A inhibitor, modulates neuronal activity in various brain regions in phMRI and EEG studies with and without ketamine challenge. *Neuroscience*, *339*, 180-190. https://doi.org/10.1016/j.neuroscience.2016.10.006, https://doi.org/10.1016/j.neuroscience.2016.10.006

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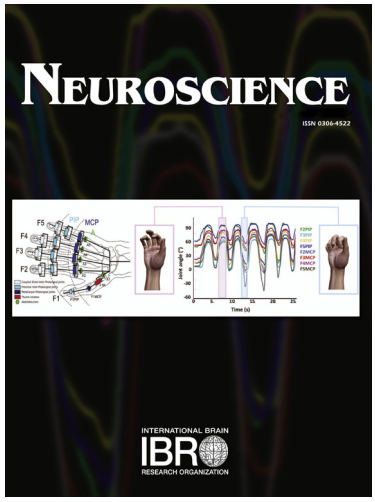

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PII: S0306-4522(16)30515-2
DOI: http://dx.doi.org/10.1016/j.neuroscience.2016.10.006
Reference: NSC 17366

To appear in: *Neuroscience*

Accepted Date: 2 October 2016

Please cite this article as: Y. Tomimatsu, D. Cash, M. Suzuki, K. Suzuki, M. Bernanos, C. Simmons, S.C.R. Williams, H. Kimura, TAK-063, a phosphodiesterase 10A inhibitor, modulates neuronal activity in various brain regions in phMRI and EEG studies with and without ketamine challenge, *Neuroscience* (2016), doi: http://dx.doi.org/10.1016/j.neuroscience.2016.10.006

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Title page

TAK-063, a phosphodiesterase 10A inhibitor, modulates neuronal activity in various brain regions in phMRI and EEG studies with and without ketamine challenge

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ABSTRACT

TAK-063 is a selective phosphodiesterase 10A (PDE10A) inhibitor that produces potent antipsychotic-like and pro-cognitive effects at 0.3 mg/kg (26% PDE10A occupancy in rats) or higher in rodents through the balanced activation of the direct and indirect pathways of striatal medium spiny neurons. In this study, we evaluated the specific binding of TAK-063 using *in vitro* autoradiography and the modulation of brain activity using pharmacological magnetic resonance imaging (phMRI) and electroencephalography (EEG). [^{3}H]TAK-063 significantly accumulated in the caudate—putamen, ventral pallidum, substantia nigra, hippocampus, and amygdala, but not in the frontal cortex, brainstem, or cerebellum in an autoradiography study using rat brain sections. [^{3}H]TAK-063 accumulation in the caudate—putamen was more than eighteen-fold higher than that in the hippocampus and amygdala. TAK-063 at 0.3 mg/kg increased the blood oxygenation level-dependent (BOLD) signal in the striatum and amygdala, and decreased it in the frontal cortex in a phMRI study with anesthetized rats. TAK-063 at 0.3 mg/kg significantly reduced the ketamine-induced increase in EEG gamma power both in awake and anesthetized rats. TAK-063 at 0.2 mg/kg (35% PDE10A occupancy in monkeys) also reduced the ketamine-induced increase in EEG gamma power in awake monkeys. In line with the EEG data, TAK-063 at 0.3 mg/kg reversed the ketamine-induced BOLD signal changes in the cortex, brainstem, and cerebellum in a phMRI study with anesthetized rats. These data suggest that TAK-063 at about 30% PDE10A occupancy modulates activities of multiple brain regions through activation of neuronal circuits in rats and monkeys.

Keywords

PDE10A, TAK-063, phMRI, EEG

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Abbreviations

Amy, amygdala; ARG, autoradiography; AUC, area under the curve; BOLD, blood oxygenation level-dependent; Bs, brainstem; cAMP, cyclic adenosine monophosphate; Cb, cerebellum; cGMP, cyclic guanosine monophosphate; CNS, central nervous system; CPu, caudate—putamen; EEG, electroencephalography; Fcx, frontal cortex; FFT, Fast Fourier Transformations; Hipp, hippocampus; ic, internal capsule; MSNs, medium spiny neurons; NMDA, *N*-methyl-$_{D}$-aspartate; PDE10A, Phosphodiesterase 10A; phMRI, pharmacological magnetic resonance imaging; PSL, photostimulated luminescence; SN, substantia nigra; VP, ventral pallidum

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Introduction

The connectivity of the corticostriatal circuit enables sensory inputs to be associated with the output functions such as motor and cognitive responses (Shepherd, 2013). The circuit consists of a cortical and a striatal component (Hersch et al., 1995, Bolam et al., 2000), and the medium spiny neurons (MSNs) in the striatum are the principal cells that receive inputs from cortical components. MSNs project in two different directions, namely the direct and indirect pathways (Gerfen and Surmeier, 2011). These two pathways are considered to have a competing effect on the striatal outputs and on the consequent modulation of thalamic and cortical functions (Silkis, 2001).

Phosphodiesterase 10A (PDE10A) is a dual-substrate enzyme that hydrolyzes both Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Fujishige et al., 1999, Soderling et al., 1999). PDE10A is highly expressed in MSNs of the mammalian striatum (Soderling and Beavo, 2000, Seeger et al., 2003, Xie et al., 2006), and regulates the output function of both the direct and indirect pathways (Siuciak et al., 2006). Dysfunction of the corticostriatal circuit has been implicated in various central nervous system (CNS) disorders including schizophrenia; thus, pharmacological inhibition of PDE10A and the resulting activation of the corticostriatal circuit could be a promising therapeutic approach for these disorders (Kehler and Nielsen, 2011, Kehler, 2013).

TAK-063 is a potent, selective, and orally active PDE10A inhibitor (Kunitomo et al., 2014, Harada et al., 2015). Similar to other PDE10A inhibitors such as MP-10 (Schmidt et al., 2008, Grauer et al., 2009), TAK-063 showed potent antipsychotic-like effects in some rodent models of schizophrenia such as MK-801-induced hyperactivity, and lower risks of side effects than that showed by the current antipsychotics (Suzuki et al., 2015). Interestingly, TAK-063, but not MP-10, showed potent antipsychotic-like effects in methamphetamine-induced hyperactivity and prepulse inhibition deficits in rodents. Characterization of underlying

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mechanisms of action revealed that TAK-063 can induce more balanced activation of the direct and indirect pathways than MP-10 did via its faster off-rate from PDE10A (Suzuki et al., 2016). In addition, TAK-063 enhanced various cognitive functions, such as recognition memory, attention, impulsivity, working memory, and executive function, in naïve rats or in the *N*-methyl-$_D$-aspartate (NMDA) receptor antagonist-induced rodent models of schizophrenia (Shiraishi et al., 2016). Thus, it is plausible that TAK-063 can modulate the function of multiple brain regions through the augmentation of striatal outputs.

In this study, we evaluated the detailed binding pattern of TAK-063 using *in vitro* autoradiography (ARG) with rat serial brain sections, and then investigated the effects of TAK-063 on brain activity by pharmacological magnetic resonance imaging (phMRI) in rats and electroencephalography (EEG) in rats and monkeys. EEG has a good temporal resolution and directly measures the neuronal activity although it has limited spatial resolution restricted to the cortical region close to the surface of the brain (Gloor, 1985). phMRI, on the other hand, has a whole brain coverage but suffers from limitation of low temporal resolution; the blood oxygenation level-dependent (BOLD) response in phMRI is only indirectly linked to a neuronal activity (Leslie and James, 2000). The three methods together therefore should be highly complementary and help to reveal a fuller picture of the effects of TAK-063 on the brain function. To gain a better insight into the pharmacological profile of TAK-063 as a drug for schizophrenia, we also investigated the effect of TAK-063 on the signal produced by ketamine, an NMDA receptor blocker, that is known to induce schizophrenia-like symptoms in multiple species (Littlewood et al., 2006b, Pinault, 2008, Hodkinson et al., 2012, Doyle et al., 2013, Gil-da-Costa et al., 2013). Herein we report preclinical evidence that TAK-063, by binding to PDE10A, can modulate the neuronal activity in multiple brain regions through activation of neuronal circuits.

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Material and methods

Animals

A total of 158 rats and 6 monkeys were included in the experiment. Male Wistar (CLEA Japan, Inc., Tokyo, Japan) and Sprague Dawley (SD) rats (Charles River Laboratories Japan, Inc., Yokohama, Japan, and Charles River, UK) were kept under standard laboratory conditions (12:12 h light/dark cycle) with food and water available *ad libitum*. Female cynomolgus monkeys (Macaca fascicularis, Keari Co., Ltd., Osaka, Japan) were kept under standard laboratory conditions and fed once daily with water available *ad libitum*. The care and use of the animals and the experimental protocols were in accordance with the guideline of Institutional Animal Care and Use Committee (Takeda Pharmaceutical Company Limited., Pharmacology Research Laboratories, Kanagawa, Japan) and UK Animals (Scientific Procedures) Act of 1986 and the Ethical Review Panel of King's College London.

Chemicals and radioligand

TAK-063 and MP-10 succinate were synthesized by Takeda Pharmaceutical Company Limited (Kanagawa, Japan) (Verhoest et al., 2009, Kunitomo et al., 2014). [^{3}H]TAK-063 (37.0 MBq/mL in ethanol) was synthesized by Sekisui Medical Co., Ltd. (Tokyo, Japan). The specific radioactivity and radiochemical purity of [^{3}H]TAK-063 were 665 GBq/mmol and 98.1%, respectively. Ketamine hydrochloride (Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan, or Tocris Bioscience, Abingdon, UK), (+)-MK-801 hydrogen maleate (Sigma-Aldrich, St. Louis, MO), propofol (Maruishi Pharmaceutical Co., Ltd., Osaka, Japan), and isoflurane (Abbott Laboratories, TX) were obtained commercially. TAK-063 was suspended in 0.5% (w/v) methylcellulose in distilled water for oral (p.o.) or 0.5% (w/v) methylcellulose in saline for intraperitoneal (i.p.) administration to rats. TAK-063 was dissolved in the following vehicle and administered intravenously (i.v.) to monkeys: 5% (v/v) N, N-dimethylacetamide,

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10% (v/v) Ethanol, 30% (v/v) PEG-400, and 55% (w/v) Sulfobutylether-β-cyclodextrin. Ketamine was dissolved in saline and administered subcutaneously (s.c.) to rats or intramuscularly (i.m.) to monkeys.

Regions of interest (ROI) of the brain

ROI were set and abbreviated as follows by referring to the brain atlas (Paxinos and Watson, 1997): frontal cortex (Fcx), caudate—putamen (CPu), ventral pallidum (VP), hippocampus (Hipp), internal capsule (ic), amygdala (Amy), substantia nigra (SN), brainstem (Bs), and cerebellum (Cb).

***In vitro* ARG with rat brain sections**

A total of 4 male SD rats were included in the experiment. *In vitro* ARG was conducted as previously described (Harada et al., 2015). The brains of SD rats were frozen, and then sagittal or serial coronal brain sections at 10.5, 7.6, 5.3, 3.8, 0.4 mm posterior and 3.2 mm anterior to bregma were cut in a cryostat (Leica Microsystems, Wetzlar, Germany). After pre-incubation twice for 5 min in buffer (50 mM Tris-HCl pH 7.5, 1.7 mM EDTA, 6 mM $MgCl_2$, 120 mM NaCl, and 0.1% BSA) at room temperature, the sections were incubated in binding buffer (50 mM Tris-HCl pH 7.5, 1.7 mM EDTA, 6 mM $MgCl_2$, 120 mM NaCl, 0.1% BSA, and 0.03% Triton X-100) containing 20 nM [^{3}H]TAK-063 for 60 min at room temperature. Adjacent sections were used for the blocking study by adding MP-10 (final concentration of 1 μM). The sections were washed twice for 5 min at 4°C in pre-incubation buffer, and then rapidly rinsed in ice-cold distilled water. The sections were dried under a stream of cool air, and were exposed to BAS IP TR 2040E imaging plates (GE Healthcare UK Ltd.) for 7 days. The imaging plates were analyzed using an image analyzer and software (FLA-7000 and Image Gauge, Fujifilm, Tokyo, Japan). Radioactivity in each ROI was

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analyzed and represented as photostimulated luminescence (PSL) values (/mm^2). The background was subtracted from the PSL values of each ROI, and the PSL values in each brain region were then averaged for each group. The PSL values in the absence and presence of 1 μM MP-10 were represented as total binding and blocked by MP-10, respectively.

Measurement of cyclic nucleotides in the rat striatum

A total of 32 male SD rats were included in the experiment. SD rats were sacrificed using an MMW-05 focused microwave irradiation system (Muromachi Kikai Co. Ltd., Tokyo, Japan) 30 min after i.p. administration of TAK-063. Striatum tissues were isolated, sonicated in 0.5 N HCl, and clarified by centrifugation. cAMP and cGMP concentrations in the supernatants were measured using enzyme immunoassay kits (Cayman Chemical Company, Ann Arbor, MI).

MK-801-induced hyperactivity in rats

A total of 28 male SD rats were included in the experiment. Locomotor activity of the rats was measured using a SUPERMEX spontaneous motor analyzer (Muromachi Kikai Co., Tokyo, Japan). SD rats were placed in locomotor chambers (length × width × height: 24 × 37 × 30 cm) for more than 60 min for habituation. Thereafter, rats were removed from each chamber and treated with either vehicle or TAK-063 (i.p.) and then quickly returned to the chamber. Rats were again removed from the chambers 30 min after administration of TAK-063 and treated with either vehicle (saline) or MK-801 (0.3 mg/kg as hydrogen maleate salt, s.c.) and then quickly transferred to the test chamber. Activity counts were recorded in successive 1-min bins, and then cumulative counts during 120 min after psychostimulant administration were calculated.

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EEG in awake rats

A total of 40 male Wistar rats were included in the experiment. Under anesthetic control, stainless steel screw electrodes were stereotaxically positioned on the prefrontal cortex (3 mm anterior and 2.5 mm lateral to bregma) with care not to rupture the dura. Reference electrode was positioned on the cerebellum. Rats were allowed to recover for a minimum of one week before electroencephalography (EEG) recording. EEG was recorded in awake, freely-moving conditions. After 5 min of baseline recording, rats were treated with vehicle, TAK-063 (0.03–3 mg/kg p.o.), or MP-10 (0.3–30 mg/kg p.o.). Ketamine (10 mg/kg s.c.) was administered 90 (for TAK-063 study) or 60 min (for MP-10 study) min after the drug (p.o.) administration. EEG was recorded for more than 150 min in total. EEG signals were filtered at 0.1–1 kHz, amplified using an AC amplifier (BBA-2208; Biotex Ltd., Kyoto, Japan), and digitized at 500 Hz using a MICRO3 interface system (Cambridge Electronic Design Ltd., Cambridge, UK). Spike 2 software (Cambridge Electronic Design Ltd., Cambridge, UK) was used to conduct Fast Fourier Transformations (FFT) analysis and to calculate the total power in the gamma (30–80 Hz) frequency. FFT values of the noise-free 32-sec epoch were used as the representative value for 5 min bin. The gamma power of the each 5 min bin was normalized to the gamma power during baseline recording, and then area under the curve (AUC) for resting (0 to 90 or 60 min before the ketamine challenge) and ketamine-induced activated (0 to 60 min after the ketamine challenge) states were calculated for the statistical data analysis.

EEG in anesthetized rats

This experiment was conducted at King's College London. A total of 22 male SD rats were included in the experiment. Under anesthetic control, monopolar surface electrodes were stereotaxically positioned on the parietal cortex (1 mm posterior and 2 mm lateral to bregma)

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with care not to rupture the dura. Reference electrode was positioned on the nasal bone. The rats were allowed to recover for a minimum of one week before EEG recordings. EEG was recorded under isoflurane anesthesia (1.5% in air/oxygen mixture 1.0/0.4 L/min). After 5 min of baseline recording, the rats were treated with vehicle or TAK-063 (0.3–3 mg/kg i.p.). Ketamine (10 mg/kg s.c.) was administered 30 min after the drug (i.p.) administration. EEG was recorded for more than 90 min in total. EEG signals were filtered at 0.1–140 Hz, amplified, and digitized online using Biopac MP100 system (BIOPAC Systems, Inc., Goleta, CA, USA). Matlab function was used to perform a power spectral analysis of EEG recording and to calculate energy in gamma (30–80 Hz) frequency range for every minute of the recording. The gamma power of the each 1 min bin was normalized to the gamma power during baseline recording, and then AUC for resting (0 to 15 min before the ketamine challenge) and ketamine-induced activated (10 to 25 min after the ketamine challenge) states were calculated for the statistical data analysis.

EEG in awake monkeys

A total of 6 female Cynomolgus monkeys were included in the experiment. Under anesthesia with propofol (60 mg/h), stainless screw electrodes were positioned on the left and right frontal, parietal, and occipital cortex, with care not to rupture the dura. The monkeys were allowed to recover for a minimum of one month before EEG recording. For EEG recording in awake states, monkeys were placed on a monkey chair. A catheter was inserted into brachial veins for continuous drug infusion. After 5 min of baseline recording and 15 min of non-treatment phase, monkeys were treated with vehicle or TAK-063 (0.2 and 0.8 mg/kg/h for 30 min) by intravenous infusion. Ketamine (1 mg/kg i.m.) was administered 10 min after the initiation of drug infusion. EEG was recorded for more than 60 min in total. Experiment was repeated using the same animal with an interval of more than 1 week. EEG signals were

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filtered (0.5–100 Hz), amplified, digitized online, and EEG montage for FFT analysis was obtained using frontal and occipital cortex electrodes (EE5518, NEC Medical Systems, Tokyo, Japan). SleepSign program (Kissei Comtec, Nagano, Japan) was used to conduct FFT analysis and to calculate the total power in the gamma (30–80 Hz) frequency. The gamma power of the each 5 min bin was normalized to the gamma power during baseline recording, and then AUC for resting (0 to 10 min before the ketamine challenge) and ketamine-induced activated (0 to 20 min after the ketamine challenge) states were calculated for the statistical data analysis.

Statistical analysis of EEG and ARG data

Data are presented as mean+standard error of the mean (SEM). Pairwise differences were analyzed using Aspin-Welch's t-test. Dose-related effects were analyzed using Bartlett's test, which was used for testing the homogeneity of variances, followed by two-tailed Williams' test (for parametric data, $P>0.05$ by Bartlett's test) or two-tailed Shirley-Williams test (for non-parametric data, $P\leq0.05$ by Bartlett's test). Data were analyzed using EXSUS (Ver.8.0.0, CAC EXICARE Corporation, Tokyo, Japan) and statistical significance was set at $P\leq0.05$.

phMRI in rats

This experiment was conducted at King's College London. A total of 32 male SD rats were included in the experiment. Pharmacological MRI was conducted on a 7 Tesla (T) horizontal bore MR scanner (Agilent, USA) using a birdcage quadrature radiofrequency coil (Magnetic Resonance Laboratories, UK). SD rats were anesthetized with isoflurane and transferred to the scanner and secured in a stereotaxic head frame. Subcutaneous and intraperitoneal cannulas were implanted for drug administration, and probes were attached for monitoring oxygen saturation, heart rate (Nonin, USA), and respiratory rate (Biopac Systems Inc, USA). A rectal probe coupled to a thermostat-controlled heating blanket was used to maintain the

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body temperature at 37±1ºC (Harvard Apparatus, USA). Anesthesia was maintained at 1.5-1.8% isoflurane (in oxygen/air 1:9, a flow of 1 L/min) throughout the experiment. Fast spin echo structural MR images were first acquired (TR=4000 ms; effective TE=60 ms, FOV 3.2 x 3.2 cm, matrix 128 x 128 x 24 voxels, 1 mm thick contiguous slices in 8 min 40 sec). The BOLD sensitive gradient echo MR sequence consisted of 56 x 32" whole brain scans obtained before TAK-063, followed by another 56 scans before ketamine and 56 scans after ketamine/vehicle injection, yielding a total imaging time for 168 brain volumes of ca. 90 minutes. The imaging parameters were TR=500 ms, multi TE of 5, 10 and 15 ms (mean TE was analyzed), flip angle 30º, FOV 3.2 x 3.2 cm, matrix 64 x 64 x 24 voxels, 1 mm thick contiguous slices.

Images were converted from native format to Analyze format using a locally written Matlab script. All preprocessing and statistical image analysis was performed by SPM8 software (UCL, London). Intra-session movement correction (6-parameter, rigid body spatial transformation) of the time series was performed using the first image of the series (co-registered to anatomical scan) as reference. A brain mask was created using the software Jim (version 5.0_10, Xinapse Systems Inc., UK), and applied to all the realigned volumes of the time series. Resulting images contained only brain tissue. Images were then spatially normalized to a local rat brain template. Individual animals were analyzed using a General Linear Model approach, which measures the correlation between the signal intensity changes and hypothetical regressors. Regressor was derived from the EEG bench experiment and consisted of 140 zeroes (0's), followed by 28 ones (1's). The level of statistical significance was set at $P<0.05$ (uncorrected). A second-level (random effects) group analysis was then performed, using the contrast images generated in the first-level analysis. To highlight the effect of the treatments, statistical parametric maps (SPMs) figures were produced with the level of statistical significance set at $P<0.05$ (uncorrected).

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Results

TAK-063 highly accumulated in striatal complex in rat brain sections and modified the phMRI-BOLD signal in multiple brain regions in anesthetized rats

We previously reported that TAK-063 specifically bound to PDE10A in the rodent brain sections (Harada et al., 2015). Thus, ARG study with [^{3}H]TAK-063 is a sensitive way to understand not only binding pattern of TAK-063 but also the distribution of PDE10A protein in rodent brain. To obtain a more precise binding pattern of TAK-063 and to determine how this consequently affects neuronal activity in various rat brain regions, we performed a detailed *in vitro* ARG using [^{3}H]TAK-063 and serial coronal brain slices at 10.5, 7.6, 5.3, 3.8, 0.4 mm posterior and 3.2 mm anterior from Bregma (Figure 1A). [^{3}H]TAK-063 radioactivity was measured in several brain regions in the presence or absence of 1 μM of MP-10, a potent PDE10A inhibitor, and was represented as a PSL value (/mm^2); the PSL values in the presence of 1 μM of MP-10 were considered as background (Figure 1B). In line with our previous results, [^{3}H]TAK-063 highly accumulated in the CPu and SN where PDE10A is highly expressed (Figure 1A, 1B, CPu: *df* (degrees of freedom) = 3.06, $t = 7.83$, $P \leq 0.05$; SN: *df* = 3.29, $t = 15.12$, $P \leq 0.05$, Aspin-Welch's test), and a weaker but significant accumulation of [^{3}H]TAK-063 was detected in the Hipp (Figure 1B, *df* = 6.00, $t = 2.45$, $P \leq 0.05$, Aspin-Welch's t-test). Moreover, in this study, [^{3}H]TAK-063 also highly accumulated in the VP and ic, and a weaker but significant accumulation was detected in the Amy (Figure 1A, 1B, VP: *df* = 3.19, $t = 17.15$, $P \leq 0.05$; i.c.: *df* = 3.96, $t = 15.35$, $P \leq 0.05$; Amy: *df* = 3.75, $t = 4.26$, $P \leq 0.05$, Aspin-Welch's t-test). The specific PSL value in the CPu was more than eighteen-fold higher than in the Hipp and Amy (CPu 221.2±25.4, Hipp 7.1±1.6, Amy 12.1±2.9).

In our previous studies with rodent models of schizophrenia, TAK-063 was efficacious for psychosis—like symptoms and cognitive deficits at 0.3 mg/kg p.o. (26% PDE10A occupancy),

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and produced a weak cataleptic response at 3 mg/kg p.o. (77% PDE10A occupancy) (Suzuki et al., 2015, Shiraishi et al., 2016). Oral administration of TAK-063 was not available for experiments performed under anesthesia. TAK-063 with i.p. administration produced up-regulation of striatal cyclic nucleotides (Figure 1C and D, cAMP: $P>0.05$ by Bartlett's test, 0.3 mg/kg, $df = 28$, $t = 2.51$, $P\leq0.05$, Williams' test; cGMP: $P>0.05$ by Bartlett's test, 0.1 mg/kg, $df = 28$, $t = 2.17$, $P\leq0.05$; 0.3 mg/kg, $df = 28$, $Z = 2.52$, $P\leq0.05$, Williams' test) and suppression of MK-801-induced hyperactivity (Figure 1E, $P\leq0.05$ by Bartlett's test, 0.1 mg/kg, $df = 20$, $t = 2.70$, $P\leq0.05$; 0.3 mg/kg, $df = 20$, $t = 3.67$, $P\leq0.05$, Shirley-Williams test) in rats. Given that the dose response effects of TAK-063 were similar between i.p. and p.o. administration (Suzuki et al., 2015), we chose to investigate the dose of 0.3 mg/kg i.p. as an efficacious dosage for our imaging studies.

In the rat phMRI study observing the pattern of the changes of the neuronal activity in a brain coronal plane, intraperitoneal administration of TAK-063 at 0.3 mg/kg induced significant increases in BOLD signal in the CPu and Amy, whereas it decreased BOLD signal in the Fcx under isoflurane anesthesia (Figure 1F). TAK-063 at 3 mg/kg i.p. induced more localized and bilateral increases in BOLD signal in the CPu and Amy, and prominent negative BOLD signal in the Bs and Cb (Figure 1G). The phMRI data in the sagittal plane showed that TAK-063 at 0.3 and 3 mg/kg i.p. produced significant increases in BOLD signal in the CPu and SN corresponding with the high specific binding of [^{3}H]TAK-063 observed in the ARG with rat sagittal brain sections. On the other hand, a negative BOLD signal was observed in brain areas such as the Fcx and Bs where the specific binding of [^{3}H]TAK-063 was not observed in the ARG study (Figure 1H). These results suggest that TAK-063 modifies the neuronal activity in brain regions beyond the localization of PDE10A in the rat brain.

TAK-063 reduced both the resting and ketamine-induced cortical EEG gamma power in

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awake rats

The *in vivo* phMRI results suggested that TAK-063 modifies neuronal activity in the cortex; thus, we assessed the effect on cortical EEG gamma power, a possible indicator of activation of neuronal assembly (Niessing et al., 2005), in awake rats. Because ketamine is reported to induce aberrant cortical activation that is similar to that associated with schizophrenia (Baldeweg et al., 1998, Lee et al., 2008) and TAK-063 showed antipsychotic-like and pro-cognitive effects in NMDA antagonist-induced rodent models of schizophrenia (Suzuki et al., 2015, Shiraishi et al., 2016), we used ketamine here to assess the effect of TAK-063.

In awake rats, oral administration of TAK-063 reduced the resting gamma power measured before ketamine challenge (Figure 2A), and the AUC analysis showed a significant reduction of the resting gamma power at 3 mg/kg p.o., but not at 0.3 mg/kg p.o. or lower (Figure 2B, $P>0.05$ by Bartlett's test, $df = 12$, $t = 4.55$, $P\leq0.05$, Williams' test). The ketamine challenge (10 mg/kg s.c.) increased the gamma power in the vehicle group (activated states, Figure 2A), and this ketamine-induced increase in gamma power was reduced by TAK-063; the AUC analysis confirmed that ketamine significantly increased the gamma power ($df = 4.54$, $t = 8.82$, $P\leq0.05$, Aspin-Welch's t-test) and that the ketamine-induced increase in gamma power was significantly suppressed by TAK-063 at both 0.3 and 3 mg/kg p.o. (Figure 2C, $P>0.05$ by Bartlett's test, 0.3 mg/kg, $df = 12$, $t = 5.23$, $P\leq0.05$; 3 mg/kg, $df = 12$, $t = 4.57$, $P\leq0.05$, Williams' test). These data suggest that TAK-063 at 0.3 mg/kg p.o. and higher suppresses the ketamine-induced aberrant change in cortical activity measured by EEG.

In our previous study, TAK-063, but not MP-10, produced potent antipsychotic-like effects in methamphetamine-induced hyperactivity and prepulse inhibition deficits in rodents (Suzuki et al., 2016). To gain some insight into the difference between TAK-063 and MP-10, we assessed the effect of MP-10 on cortical EEG gamma power. Similar to TAK-063, oral administration of MP-10 reduced the resting gamma power measured before ketamine

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challenge (Figure 2D), and the AUC analysis showed a significant reduction of the resting gamma power at 30 mg/kg p.o. (Figure 2E, $P>0.05$ by Bartlett's test, $df = 12$, $t = 3.74$, $P\leq0.05$, Williams' test). On the other hand, MP-10 at dose up to 30 mg/kg p.o. did not significantly reduce the ketamine-induced increase in gamma power (Figure 2F).

TAK-063 reduced the ketamine-induced, but not resting, cortical EEG gamma power in awake monkeys

To understand the possible species differences in TAK-063-induced EEG changes, we next examined the effect of TAK-063 on cortical EEG before and after ketamine challenge in monkeys. In our previous monkey study, intravenous administration of TAK-063 at 0.2 and 0.8 mg/kg produced 35% and 73% PDE10A occupancy, respectively (Takano et al., 2015, Takano et al., 2016). In rodents, TAK-063 at around 30% occupancy produced potent antipsychotic-like and pro-cognitive effects (Suzuki et al., 2015, Shiraishi et al., 2016), thus 0.2 mg/kg i.v. was selected as a possible efficacious dosage in monkeys. In awake monkeys, TAK-063 at 0.2 and 0.8 mg/kg i.v. did not affect the resting gamma power (Figure 3A, B). The ketamine challenge (1 mg/kg i.m.) acutely increased the gamma power and this was suppressed by TAK-063 (Figure 3A); the AUC analysis confirmed that ketamine significantly increased the gamma power ($df = 9.87$, $t = 8.05$, $P\leq0.05$, Aspin-Welch's t-test) and that TAK-063 at both 0.2 and 0.8 mg/kg i.v. significantly reduced this increase (Figure 3C, $P>0.05$ by Bartlett's test, 0.2 mg/kg, $df = 15$, $t = 3.68$, $P\leq0.05$; 0.8 mg/kg, $df = 15$, $t = 5.85$, $P\leq0.05$, Williams' test,). These data, together with the EEG data measured in rats (Figure 2), suggest that TAK-063 suppresses the ketamine-induced activation of cortical neurons at similar occupancy (~30%) in both rats and monkeys.

Ketamine-induced increase in cortical gamma power and its reduction by TAK-063 in

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rats were preserved under isoflurane anesthesia

PhMRI study using rats should be performed under anesthesia because of the ethical requirements and the inability to keep the animals completely still and stress-free in the scanner. To further characterize the effect of TAK-063 on ketamine-induced cortical modulation using phMRI, we decided to investigate the effects of isoflurane anesthesia on the EEG parameters of interest. Because the experiment was conducted under anesthesia, TAK-063 was administered intraperitoneally. In isoflurane anesthetized rats, i.p. administration of TAK-063 continued to suppress the ketamine-induced (10 mg/kg s.c.) increases in gamma power without affecting the resting gamma power (Figure 4A, B). The AUC analysis confirmed that ketamine significantly increased the gamma power ($df = 5.57$, $t = 4.40$, $P \leq 0.05$, Aspin-Welch's t-test) and that TAK-063 at 0.3 and 3 mg/kg i.p. significantly reduced this increase (Figure 4C, $P > 0.05$ by Bartlett's test, 0.3 mg/kg, $df = 14$, $t = 2.43$, $P \leq 0.05$; 3 mg/kg, $df = 14$, $t = 3.70$, $P \leq 0.05$, Williams' test). Thus, isoflurane was selected as an anesthesia to further investigate the effects of TAK-063 on ketamine-induced cortical modulation by phMRI.

TAK-063 reversed the ketamine-induced changes in phMRI-BOLD signals in multiple brain regions in rats

The ketamine challenge (10 mg/kg s.c.) produced an increase in BOLD signal in many brain regions, including the cortex, CPu, Hipp, and Amy. Ketamine, on the contrary, decreased the signal in the Bs, Cb, and hypothalamus (Figure 5A, D). TAK-063 at 0.3 mg/kg i.p. suppressed the ketamine-induced increase in BOLD signal in the Hipp and cortical areas such as the cingulate (frontal) and retrosplenial (parietal) cortex. On the other hand, TAK-063 at 0.3 mg/kg i.p. reversed the ketamine-induced decrease in BOLD signal in the Bs, Cb, and hypothalamus. In addition, TAK-063 at 0.3 mg/kg i.p. increased the signal in the thalamus and

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hypothalamus and augmented the ketamine-induced increases in the CPu (Figure 5B, E). TAK-063 at 3 mg/kg i.p. suppressed the ketamine-induced cortical signals more strongly than at 0.3 mg/kg i.p., with a more robust and localized activation of the CPu and Amy. However, TAK-063 at 3 mg/kg i.p. did not change the activity in the thalamic and hypothalamic areas (Figure 5C, F). These data suggest that TAK-063 at the efficacious dosage modulates the ketamine-induced BOLD signal changes, while some of the effects disappear with overdosing.

Discussion

In our previous ARG study with brain sections of wild-type (WT) and PDE10A knock-out (KO) mice, [^{3}H]TAK-063 showed PDE10A-specific accumulation; thus, ARG study with [^{3}H]TAK-063 is a sensitive way to understand the localization of PDE10A protein (Harada et al., 2015). *In vitro* ARG study with a series of rat coronal brain sections demonstrated the high and significant accumulation of [^{3}H]TAK-063 in the caudate—putamen, ventral pallidum, internal capsule, and substantia nigra, and low but significant accumulation in the hippocampus and amygdala (Figure 1). A phMRI study measuring cerebral BOLD signal responses produced by TAK-063 alone demonstrated that TAK-063 at 0.3 mg/kg apparently activated those brain areas in anesthetized rats (Figure 1). These data suggest that TAK-063 modulates activities of multiple brain regions through the inhibition of PDE10A and the resulting activation of neuronal circuits.

We recently reported that TAK-063 improved recognition memory assessed by novel object recognition task and attention and impulsivity assessed by five-choice serial reaction time task in naive rats. In addition, we reported that TAK-063 attenuated phencyclidine (PCP)-induced spatial working memory deficits assessed by Y-maze test and MK-801–induced spatial working memory deficits assessed by the eight-arm radial maze task in rats. TAK-063 also

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ameliorated disturbed executive function in subchronic PCP-treated rats assessed by attentional set-shifting task (Shiraishi et al., 2016). These cognitive functions may be associated with various brain regions including the frontal cortex and retrosplenial cortex. Moreover, the neuronal activity in those regions can be modified by ketamine (Littlewood et al., 2006a); thus, we decided to further characterize the modulatory effects of TAK-063 on the neuronal activity after ketamine challenge in various brain regions.

The reduction of the ketamine-induced cortical activity by TAK-063 at about 30% PDE10A occupancy was observed in the EEG gamma power analysis in both awake rats and monkeys (Figures 2 and 3), suggesting that TAK-063 can indeed modulate cortical function at a similar occupancy (~30%) beyond species. We next tried to assess the modulation of neuronal activity in a whole brain by using phMRI. Under anesthesia, phMRI responses to drugs of interest can vary compared with the awake state (Luo et al., 2007). However, by using isoflurane anesthesia, we confirmed the preserved effect of TAK-063 on the ketamine-induced increase in cortical gamma power (Figure 4). Thus, we expected to detect phMRI signals associated with anti-ketamine effects of TAK-063 under the experimental conditions.

TAK-063 at 0.3 mg/kg suppressed the ketamine-induced neuronal activation in the cortex and reversed the ketamine-induced deactivation in the brainstem, cerebellum, and hypothalamus (Figure 5). Within cortical areas, modulation of neuronal activity by TAK-063 0.3 mg/kg i.p. was observed in prefrontal and retrosplenial cortex (Figures 5). The prefrontal cortex is implicated in cognitive functions like attention, impulsivity, and executive function (Birrell and Brown, 2000, Maddux and Holland, 2011), and the retrosplenial cortex is, by interacting with the hippocampus, implicated in the recognition and working memory functions (Haijima and Ichitani, 2008, Cohen and Stackman, 2015). The improvement of cognitive function produced by TAK-063 (Shiraishi et al., 2016) may result from the modulation of neuronal activity of these brain regions, although further studies are needed to elucidate the relationship

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between the brain activity and pro-cognitive effects produced by TAK-063.

We also investigated the consequences of overdosing by comparing the effects of TAK-063 at 0.3 and 3 mg/kg. Although, overall, TAK-063 at 0.3 and 3 mg/kg produced similar responses in both phMRI and EEG, some differences were found. Namely, TAK-063 at 3 mg/kg decreased the BOLD signal in the brainstem and cerebellum (Figure 1) and reduced EEG gamma power in resting states of awake rats (Figure 2). In addition, TAK-063 at 3 mg/kg did not reverse the ketamine-induced phMRI-BOLD signal changes in the brainstem, cerebellum, thalamus, and hypothalamic areas (Figure 5). Further studies are needed to elucidate the relationship between brain activity, efficacy and the unwanted side effects produced by overdosing.

Different from TAK-063, MP-10 did not significantly reduce the ketamine-induced increase in gamma power in rats (Figure 2). In our previous study, TAK-063, but not MP-10, showed potent antipsychotic-like effects in methamphetamine-induced hyperactivity and prepulse inhibition deficits in rodents (Suzuki et al., 2016). We hypothesize that the excess activation of the direct pathway by MP-10 may be associated with its lack of efficacy in these paradigms. In fact, excess activation of the direct pathway by a dopamine D1 receptor agonist SKF82958 canceled antipsychotic-like effects of a dopamine D2 receptor antagonist haloperidol in methamphetamine-induced hyperactivity in rats (Suzuki et al., 2016). TAK-063 has a faster off-rate than MP-10, and thus, shows higher sensitivity to binding inhibition by cyclic nucleotides. Cyclic AMP levels in the direct pathway of MSNs seemed to be higher than those in the indirect pathway of MSNs. Therefore, TAK-063 and MP-10 activated the indirect pathway to a similar extent, while MP-10 caused greater activation of the direct pathway than TAK-063 did (Suzuki et al., 2016). This difference in the neuronal activation pattern may relate to the observed differences in the reduction of the ketamine-induced increase in gamma power (Figure 2).

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In summary, we demonstrated that TAK-063 at 0.3 mg/kg i.p. in rats and at 0.2 mg/kg i.v. in monkeys modified the activity of various brain regions beyond the localization of PDE10A protein. We have previously demonstrated that TAK-063 at 0.3 mg/kg p.o. produced antipsychotic effects and cognitive improvements in animal models of schizophrenia (Suzuki et al., 2015, Shiraishi et al., 2016, Suzuki et al., 2016). TAK-063 at the efficacious dosage may have the potential to modulate multiple brain circuits, including the corticostriatal circuit, via the specific binding to PDE10A, and can provide an opportunity to treat CNS disorders associated with dysfunction in multiple brain regions. Both EEG and phMRI are highly translational methods that can be performed in both animals and humans. The EEG and phMRI data we show here would be useful to bridge pre-clinical and clinical studies. TAK-063 is currently in clinical development in patients with schizophrenia (ClinicalTrials.gov Identifiers: NCT02477020).

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Conflicts of interest

The experiments conducted at King's College London were funded by Takeda Pharmaceutical Company Limited. Mr Yoshiro Tomimatsu, Dr Motohisa Suzuki, Dr Kazunori Suzuki, and Dr Haruhide Kimura are employees of Takeda Pharmaceutical Company Limited and declare no other competing financial interests. Dr Diana Cash, Dr Michel Bernanos, Dr Camilla Simmons, and Dr Steven C.R. Williams have no conflict of interest.

Acknowledgements

We wish to express our sincere thanks to Drs Sarah Almond and Hird Nicholas for their support and helpful discussions. We also wish to express our sincere thanks to Dr Aisling Koning for her helpful editing of the manuscript.

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ACCEPTED MANUSCRIPT

References

Baldeweg T, Spence S, Hirsch SR, Gruzelier J (1998) Gamma-band electroencephalographic oscillations in a patient with somatic hallucinations. Lancet 352:620-621.

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci 20:4320-4324.

Bolam JP, Hanley JJ, Booth PA, Bevan MD (2000) Synaptic organisation of the basal ganglia. J Anat 196 (Pt 4):527-542.

Cohen SJ, Stackman RW, Jr. (2015) Assessing rodent hippocampal involvement in the novel object recognition task. A review. Behav Brain Res 285:105-117.

Doyle OM, De Simoni S, Schwarz AJ, Brittain C, O'Daly OG, Williams SC, Mehta MA (2013) Quantifying the attenuation of the ketamine pharmacological magnetic resonance imaging response in humans: a validation using antipsychotic and glutamatergic agents. J Pharmacol Exp Ther 345:151-160.

Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K (1999) Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J Biol Chem 274:18438-18445.

Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34:441-466.

Gil-da-Costa R, Stoner GR, Fung R, Albright TD (2013) Nonhuman primate model of schizophrenia using a noninvasive EEG method. Proc Natl Acad Sci U S A 110:15425-15430.

Gloor P (1985) Neuronal generators and the problem of localization in electroencephalography: application of volume conductor theory to electroencephalography. J Clin Neurophysiol 2:327-354.

Grauer SM, Pulito VL, Navarra RL, Kelly MP, Kelley C, Graf R, Langen B, Logue S,

ACCEPTED MANUSCRIPT

Brennan J, Jiang L, Charych E, Egerland U, Liu F, Marquis KL, Malamas M, Hage T, Comery TA, Brandon NJ (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J Pharmacol Exp Ther 331:574-590.

Haijima A, Ichitani Y (2008) Anterograde and retrograde amnesia of place discrimination in retrosplenial cortex and hippocampal lesioned rats. Learn Mem 15:477-482.

Harada A, Suzuki K, Kamiguchi N, Miyamoto M, Tohyama K, Nakashima K, Taniguchi T, Kimura H (2015) Characterization of binding and inhibitory properties of TAK-063, a novel phosphodiesterase 10A inhibitor. PLoS One 10:e0122197.

Hersch SM, Ciliax BJ, Gutekunst CA, Rees HD, Heilman CJ, Yung KK, Bolam JP, Ince E, Yi H, Levey AI (1995) Electron microscopic analysis of D1 and D2 dopamine receptor proteins in the dorsal striatum and their synaptic relationships with motor corticostriatal afferents. J Neurosci 15:5222-5237.

Hodkinson DJ, de Groote C, McKie S, Deakin JF, Williams SR (2012) Differential Effects of Anaesthesia on the phMRI Response to Acute Ketamine Challenge. Br J Med Med Res 2:373-385.

Kehler J (2013) Phosphodiesterase 10A inhibitors: a 2009 - 2012 patent update. Expert Opin Ther Pat 23:31-45.

Kehler J, Nielsen J (2011) PDE10A inhibitors: novel therapeutic drugs for schizophrenia. Curr Pharm Des 17:137-150.

Kunitomo J, Yoshikawa M, Fushimi M, Kawada A, Quinn JF, Oki H, Kokubo H, Kondo M, Nakashima K, Kamiguchi N, Suzuki K, Kimura H, Taniguchi T (2014) Discovery of 1-[2-fluoro-4-(1H-pyrazol-1-yl)phenyl]-5-methoxy-3-(1-phenyl-1H-pyrazol-5-yl)pyridazin-4(1H)-one (TAK-063), a highly potent, selective, and orally active phosphodiesterase 10A (PDE10A) inhibitor. J Med Chem 57:9627-9643.

ACCEPTED MANUSCRIPT

Lee SH, Choo JS, Im WY, Chae JH (2008) Nonlinear analysis of electroencephalogram in schizophrenia patients with persistent auditory hallucination. Psychiatry Investig 5:115-120.

Leslie RA, James MF (2000) Pharmacological magnetic resonance imaging: a new application for functional MRI. Trends Pharmacol Sci 21:314-318.

Littlewood CL, Cash D, Dixon AL, Dix SL, White CT, O'Neill MJ, Tricklebank M, Williams SC (2006a) Using the BOLD MR signal to differentiate the stereoisomers of ketamine in the rat. Neuroimage 32:1733-1746.

Littlewood CL, Jones N, O'Neill MJ, Mitchell SN, Tricklebank M, Williams SC (2006b) Mapping the central effects of ketamine in the rat using pharmacological MRI. Psychopharmacology (Berl) 186:64-81.

Luo F, Li Z, Treistman SN, Kim YR, King JA, Fox GB, Ferris CF (2007) Confounding effects of volatile anesthesia on CBV assessment in rodent forebrain following ethanol challenge. J Magn Reson Imaging 26:557-563.

Maddux JM, Holland PC (2011) Effects of dorsal or ventral medial prefrontal cortical lesions on five-choice serial reaction time performance in rats. Behav Brain Res 221:63-74.

Niessing J, Ebisch B, Schmidt KE, Niessing M, Singer W, Galuske RA (2005) Hemodynamic signals correlate tightly with synchronized gamma oscillations. Science 309:948-951.

Paxinos G, Watson C (1997) The Rat Brain in Stereotaxic Coordinates Compact Third Edition. Academic Press.

Pinault D (2008) N-methyl d-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. Biol Psychiatry 63:730-735.

Schmidt CJ, Chapin DS, Cianfrogna J, Corman ML, Hajos M, Harms JF, Hoffman WE, Lebel LA, McCarthy SA, Nelson FR, Proulx-LaFrance C, Majchrzak MJ, Ramirez AD,

ACCEPTED MANUSCRIPT

Schmidt K, Seymour PA, Siuciak JA, Tingley FD, 3rd, Williams RD, Verhoest PR, Menniti FS (2008) Preclinical characterization of selective phosphodiesterase 10A inhibitors: a new therapeutic approach to the treatment of schizophrenia. J Pharmacol Exp Ther 325:681-690.

Seeger TF, Bartlett B, Coskran TM, Culp JS, James LC, Krull DL, Lanfear J, Ryan AM, Schmidt CJ, Strick CA, Varghese AH, Williams RD, Wylie PG, Menniti FS (2003) Immunohistochemical localization of PDE10A in the rat brain. Brain Res 985:113-126.

Shepherd GM (2013) Corticostriatal connectivity and its role in disease. Nat Rev Neurosci 14:278-291.

Shiraishi E, Suzuki K, Harada A, Suzuki N, Kimura H (2016) The Phosphodiesterase 10A Selective Inhibitor TAK-063 Improves Cognitive Functions Associated with Schizophrenia in Rodent Models. J Pharmacol Exp Ther 356:587-595.

Silkis I (2001) The cortico-basal ganglia-thalamocortical circuit with synaptic plasticity. II. Mechanism of synergistic modulation of thalamic activity via the direct and indirect pathways through the basal ganglia. Biosystems 59:7-14.

Siuciak JA, McCarthy SA, Chapin DS, Fujiwara RA, James LC, Williams RD, Stock JL, McNeish JD, Strick CA, Menniti FS, Schmidt CJ (2006) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: evidence for altered striatal function. Neuropharmacology 51:374-385.

Soderling SH, Bayuga SJ, Beavo JA (1999) Isolation and characterization of a dual-substrate phosphodiesterase gene family: PDE10A. Proc Natl Acad Sci U S A 96:7071-7076.

Soderling SH, Beavo JA (2000) Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. Curr Opin Cell Biol 12:174-179.

Suzuki K, Harada A, Shiraishi E, Kimura H (2015) In vivo pharmacological characterization

ACCEPTED MANUSCRIPT

of TAK-063, a potent and selective phosphodiesterase 10A inhibitor with antipsychotic-like activity in rodents. J Pharmacol Exp Ther 352:471-479.

Suzuki K, Harada A, Suzuki H, Miyamoto M, Kimura H (2016) TAK-063, a PDE10A Inhibitor with Balanced Activation of Direct and Indirect Pathways, Provides Potent Antipsychotic-Like Effects in Multiple Paradigms. Neuropsychopharmacology 41:2252-2262.

Takano A, Stepanov V, Gulyas B, Nakao R, Amini N, Miura S, Kimura H, Taniguchi T, Halldin C (2015) Evaluation of a novel PDE10A PET radioligand, [(11) C]T-773, in nonhuman primates: brain and whole body PET and brain autoradiography. Synapse 69:345-355.

Takano A, Stepanov V, Nakao R, Amini N, Gulyas B, Kimura H, Halldin C (2016) Brain PET measurement of PDE10A occupancy by TAK-063, a new PDE10A inhibitor, using [(11) C]T-773 in nonhuman primates. Synapse 70:253-263.

Verhoest PR, Chapin DS, Corman M, Fonseca K, Harms JF, Hou X, Marr ES, Menniti FS, Nelson F, O'Connor R, Pandit J, Proulx-Lafrance C, Schmidt AW, Schmidt CJ, Suiciak JA, Liras S (2009) Discovery of a novel class of phosphodiesterase 10A inhibitors and identification of clinical candidate 2-[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)-phenoxymethyl]-quinoline (PF-2545920) for the treatment of schizophrenia. J Med Chem 52:5188-5196.

Xie Z, Adamowicz WO, Eldred WD, Jakowski AB, Kleiman RJ, Morton DG, Stephenson DT, Strick CA, Williams RD, Menniti FS (2006) Cellular and subcellular localization of PDE10A, a striatum-enriched phosphodiesterase. Neuroscience 139:597-607.

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Figure captions

Figure 1 Comparison between [^{3}H]TAK-063 accumulation in autoradiography and phMRI-BOLD signals induced by TAK-063. **A.** Typical autoradiogram data showing the high [^{3}H]TAK-063 radioactivity in brain areas including caudate—putamen (CPu), ventral pallidum (VP), internal capsule (ic), and substantia nigra (SN). Numbers indicate the posterior (-) and anterior (+) stereotaxic position (mm) from the bregma. **B.** Regions of interest (ROI) analysis with or without MP-10. MP-10 significantly decreased the PSL values in the CPu, VP, hippocampus (Hipp), ic, amygdala (Amy), and SN. TAK-063 elevated cAMP (**C**) and cGMP (**D**) levels in the rat striatum 30 min after i.p administration. **E.** TAK-063 suppressed the MK-801-induced (0.3 mg/kg s.c.) hyperactivity in rats. **F.** Coronal section statistical parametric mapping (SPM) figures of the phMRI-BOLD with TAK-063 at 0.3 mg/kg. TAK-063 at 0.3 mg/kg produced prominent activation of the CPu and Amy, and some negative changes in the frontal cortex (Fcx) including medial part. **G.** Coronal section SPM figures of the phMRI-BOLD with TAK-063 at 3 mg/kg. TAK-063 at 3 mg/kg produced localized and bilateral increases in BOLD signals in the CPu and Amy and prominent negative BOLD signals in the brainstem (Bs) and cerebellum (Cb). **H.** Sagittal section SPM figures of the phMRI-BOLD with TAK-063. TAK-063 at 0.3 and 3 mg/kg produced significant increases in BOLD signals in the CPu and SN corresponding with the high specific binding of [^{3}H]TAK-063 observed in ARG with rat sagittal brain sections. Data are presented as means+SEMs. n=4 for each group in autoradiography study, n=7–9 for measurement of cyclic nucleotide study, n=4–6 for locomotor study, n=8 for TAK-063 0.3 mg/kg, n=10 for TAK-063 3 mg/kg in phMRI study. * $P \leq 0.05$ by Aspin-Welch's t-test, $^{\#}$ $P \leq 0.05$ versus vehicle by Williams' test. § $P \leq 0.05$ versus vehicle + MK-801 group by Shirley–Williams test. ARG, autoradiography; BOLD, blood-oxygen-level dependent; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; Cont, control; i.p., intraperitoneal;

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MB, midbrain; PFC, prefrontal cortex; phMRI, pharmacological magnetic resonance imaging; PSL; photostimulated luminescence; s.c., subcutaneous, SEMs, standard error of the means; Veh: vehicle.

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Figure 2 Effects of TAK-063 and MP-10 on EEG gamma power analyzed by Fast Fourier Transformations (FFT) in awake rats with or without ketamine challenge. **A.** Experimental scheme is shown on top of the figure. TAK-063 reduced the gamma power in the resting states. TAK-063 also reduced the gamma power in the ketamine-induced activated states. The timing of ketamine administration is indicated as 0 min. **B.** TAK-063 at 3 mg/kg significantly reduced the AUC of the gamma power in resting states. **C.** Ketamine significantly increased the AUC of gamma power, and TAK-063 significantly reduced the ketamine-induced increase in AUC of the gamma power. **D.** Experimental scheme is shown on top of the figure. MP-10 reduced the gamma power in the resting states. The timing of ketamine administration is indicated as 0 min. **E.** MP-10 at 30 mg/kg significantly reduced the AUC of the gamma power in resting states. **F.** Ketamine significantly increased the AUC of gamma power; however, MP-10 did not significantly reduce the ketamine-induced increase in AUC of the gamma power. Data are presented as means+SEMs (n=4 for each group). $^{*}P \leq 0.05$ by Aspin-Welch's t-test, $^{\#}P \leq 0.05$ by Williams' test, AUC, area under the curve; EEG, electroencephalogram; Ket -, without ketamine; Ket +, with ketamine; MP, MP-10; p.o., oral; s.c., subcutaneous; SEMs, standard error of the means; TAK, TAK-063; Veh, vehicle.

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Figure 3 Effects of TAK-063 on EEG gamma power analyzed by Fast Fourier Transformations (FFT) in awake monkeys with or without ketamine challenge. **A.** Experimental scheme is shown on top of the figure. TAK-063 did not modify the gamma power in the resting states, while it reduced the gamma power in the ketamine-induced activated states. The timing of ketamine administration is indicated as 0 min. **B.** TAK-063 did not affect the AUC of the gamma power in resting states. **C.** Ketamine significantly increased the AUC of the gamma power, and TAK-063 significantly suppresses the ketamine-induced increase in AUC of the gamma power. Data are presented as means+SEMs (n=6 for each group). $^{*}P \leq 0.05$ by Aspin-Welch's t-test, $^{\#}P \leq 0.05$ by Williams' test, AUC, area under the curve; EEG, electroencephalogram; i.v., intravenous; i.m., intramuscular; Ket -, without ketamine; Ket +, with ketamine; SEMs, standard error of the means; TAK, TAK-063; Veh, vehicle.

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Figure 4 Effects of TAK-063 on EEG gamma power analyzed by Fast Fourier Transformations (FFT) in isoflurane anesthetized rats with or without ketamine challenge. **A.** Experimental scheme is shown on top of the figure. TAK-063 did not modify the EEG gamma power in the resting states, while it reduced the ketamine-induced increase in EEG gamma power under isoflurane anesthetized conditions. The timing of ketamine administration is indicated as 0 min. **B.** TAK-063 alone did not affect the AUC of gamma power. **C.** The ketamine-induced increase in gamma power was significantly reduced by TAK-063. Data are presented as means+SEMs. n=4 for vehicle without ketamine, n=6 for vehicle + ketamine and TAK-063 3 mg/kg + ketamine, n=5 for TAK-063 0.3 mg/kg + ketamine. $^{*}P \leq 0.05$ by Aspin-Welch's t-test, $^{\#}P \leq 0.05$ by Williams' test, AUC, area under the curve; EEG, electroencephalogram; i.p, intraperitoneal; Ket -, without ketamine; Ket +, with ketamine; s.c., subcutaneous; SEMs, standard error of the means; TAK, TAK-063; Veh, vehicle.

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Figure 5 Effects of TAK-063 on the ketamine-induced changes in phMRI-BOLD signal under isoflurane anesthetized conditions shown by statistical parametric maps (SPM) figures. **A–C.** Significant changes in the phMRI-BOLD signal produced by ketamine with or without TAK-063 are depicted on a rat brain atlas (Paxinos and Watson, 1997). Red letters indicate activation and blue letters indicate deactivation of the phMRI-BOLD signals. **D.** Ketamine challenge produced a widespread activation in the cortex, hippocampus, and striatum/amygdaloid complex; negative BOLD-contrast changes were observed in the brainstem, cerebellum, and hypothalamus. **E.** TAK-063 at 0.3 mg/kg suppressed the twoin the brainstem, cerebellum, and hypothalamus. In addition, TAK-063-induced increase in activation was observed in the thalamic and subthalamic areas, and the striatum. **F.** TAK-063 at 3 mg/kg produced a stronger suppression of the cortical activation, and a more robust and localized activation of the striatum/amygdaloid complex. *n*=8 for vehicle + ketamine, *n*=8 for TAK-063 0.3 mg/kg + ketamine, *n*=10 for TAK-063 3 mg/kg + ketamine. BOLD, blood oxygenation level dependent; phMRI, pharmacological magnetic resonance imaging.

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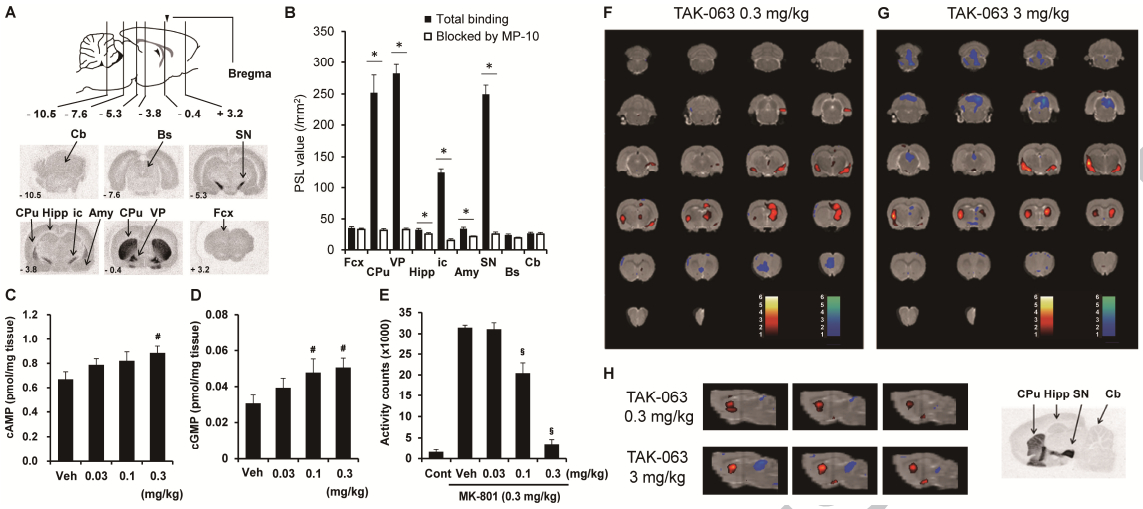

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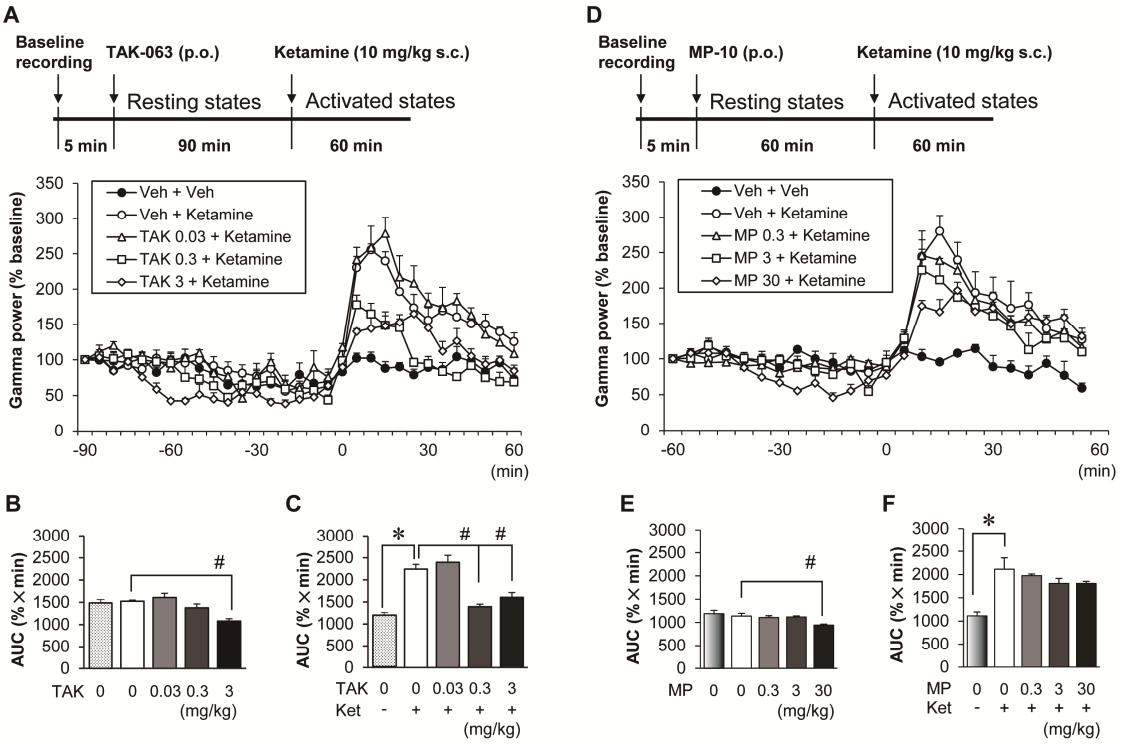

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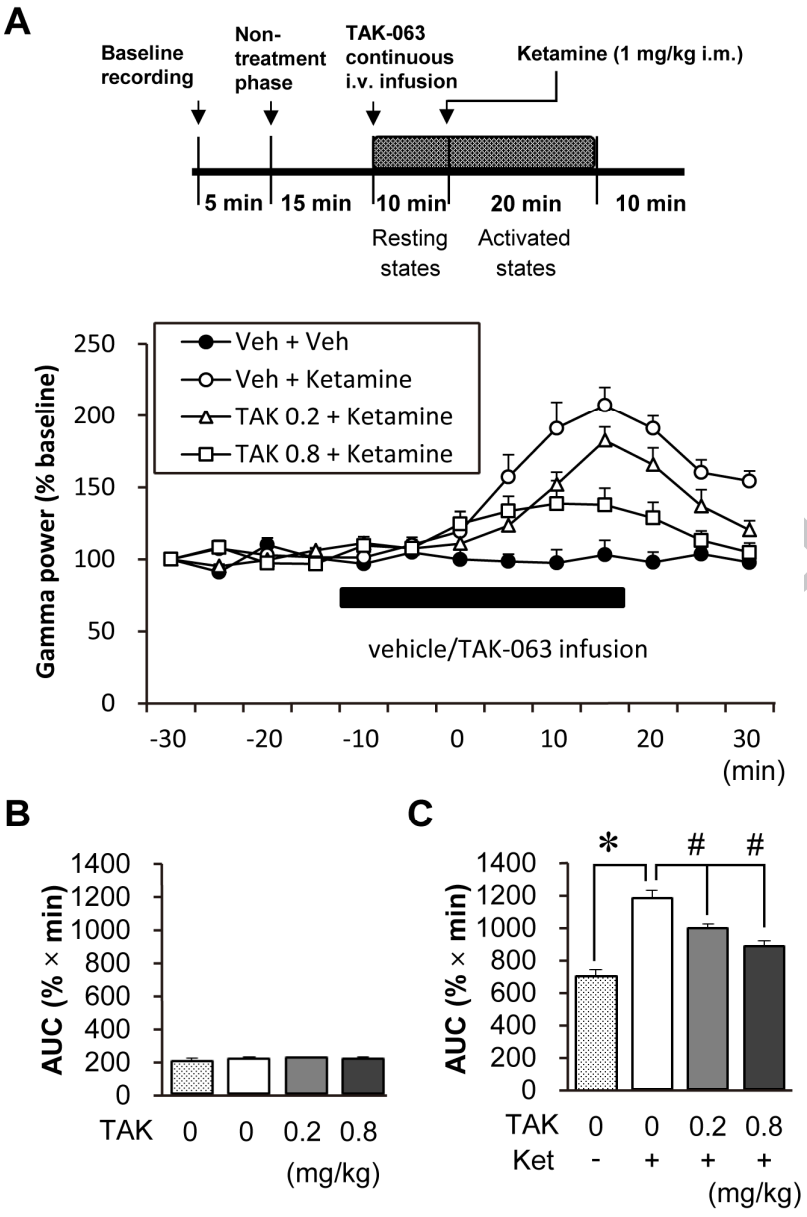

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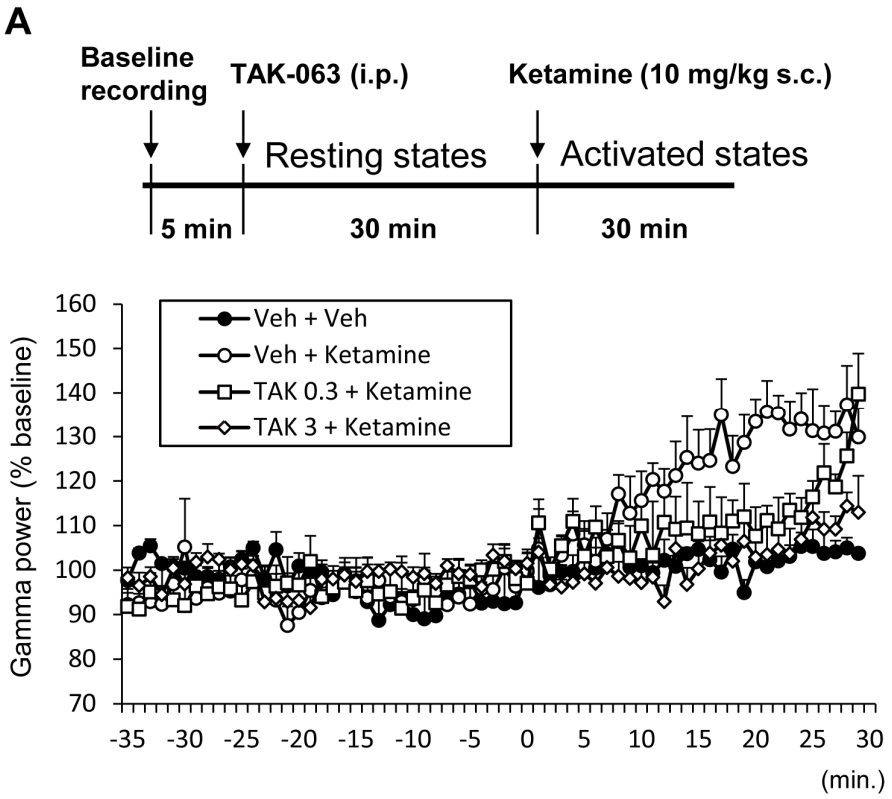

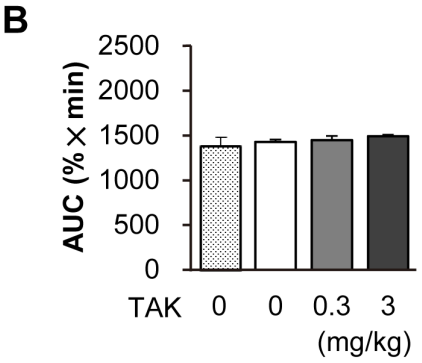

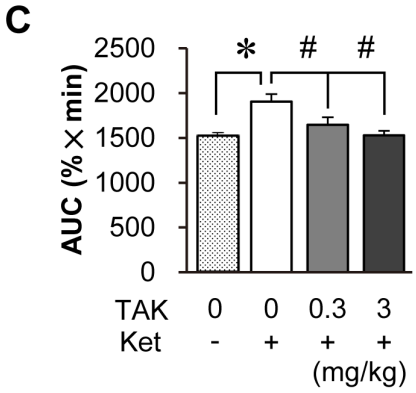

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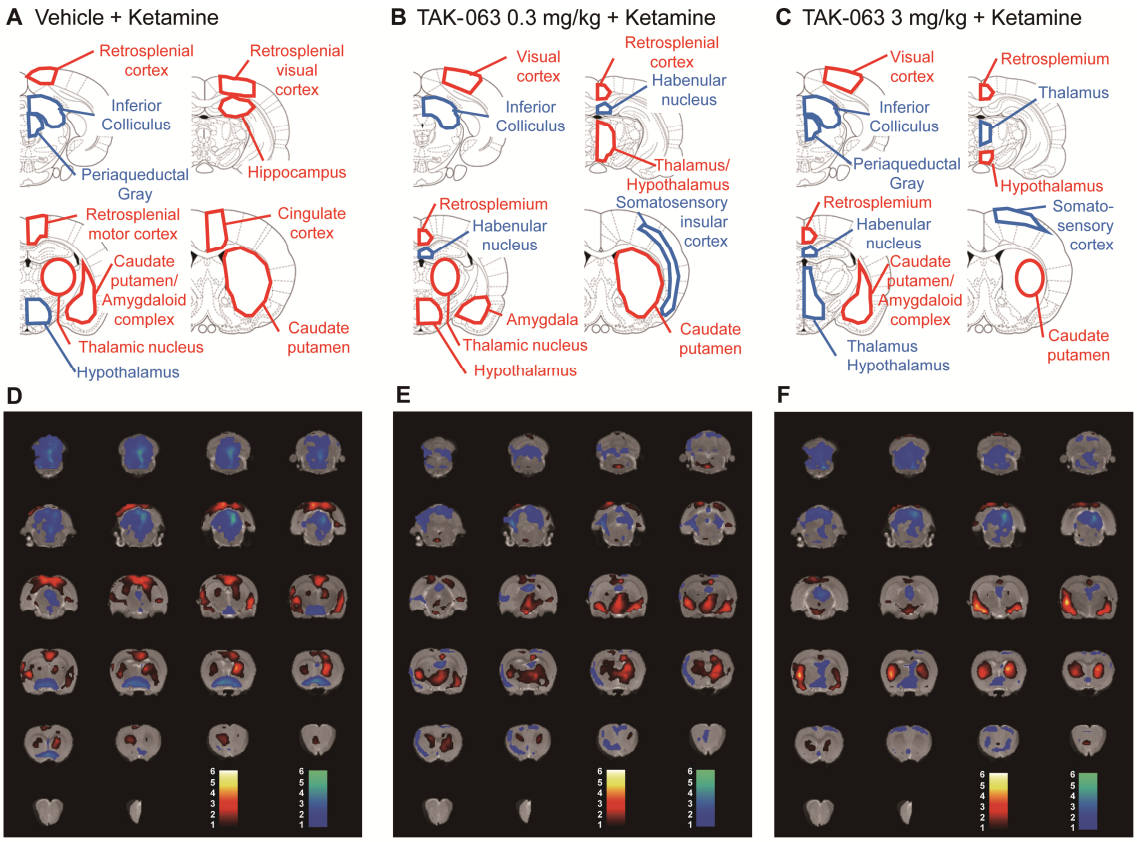

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Highlights

- [^{3}H]TAK-063 highly accumulated in the striatum and substantia nigra.
- TAK-063 modified the phMRI-BOLD signal beyond the localization of PDE10A in rats.
- TAK-063 suppressed the ketamine-induced cortical EEG activation in rats and monkeys.
- TAK-063 modulated ketamine-induced BOLD changes in various brain regions in rats.

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